Available online at www.sciencedirect.com
SCIENCE DIRECT®
Life Sciences 73 (2003) 823–847
Life Sciences
www.elsevier.com/locate/lifescie

Minireview

The role of melanocortins and their receptors in inflammatory processes, nerve regeneration and nociception

Katarzyna Starowicz[a], Barbara Przewłocka[b,*]

[a] *International Institute of Molecular and Cell Biology UNESCO/PAN, 4 Ks. Trojdena Street, 02-109 Warsaw, Poland*
[b] *Department of Molecular Neuropharmacology, Institute of Pharmacology, 12 Smetna Street, 31-343 Kraków, Poland*

Received 6 August 2002; accepted 14 January 2003

Abstract

The melanocortins are a family of bioactive peptides derived from proopiomelanocortin. Those peptides, included among hormones and comprising ACTH, α-MSH, β-MSH and γ-MSH, are best known mainly for their physiological effects, such as the control of skin pigmentation by α-MSH, and ACTH effects on pigmentation and steroidogenesis. Melanocortins are released in various sites in the central nervous system and in peripheral tissues, and participate in the regulation of multiple physiological functions. They are involved in grooming behavior, food intake and thermoregulation processes, and can also modulate the response of the immune system in inflammatory states. Research of the past decade provided evidence that melanocortins could elicit their diverse biological effects by binding to a distinct family of G protein-coupled receptors with seven transmembrane domains. To date, five melanocortin receptor genes have been cloned and characterized. Those receptors differ in their tissue distribution and in their ability to recognize various melanocortins. These advances have opened up new horizons for exploring the significance of melanocortins, their ligands and their receptors for a variety of important physiological functions. We reviewed the origin of MSH peptides, the function and distribution of melanocortin receptors and their endogenous and exogenous ligands and the role of melanocortins and their receptors in inflammatory processes, nerve regeneration and nociception. Moreover, we analyzed their interaction with opioid peptides and finally, we discussed the postulated role of the melanocortin system in pain transmission at the spinal cord level.

Keywords: Adrenocorticotropic hormone; α-melanocyte stimulating hormone; Central nervous system; Melanocortin receptor; Proopiomelanocortin

* Corresponding author. Tel.: +48-12-6623398; fax: +48-12-6374500.
E-mail address: przebar@if-pan.krakow.pl (B. Przewłocka).

0024-3205/03/$ - see front matter
doi:10.1016/S0024-3205(03)00349-7

Introduction

The melanocortin peptides, which include adrenocorticotropic hormone (ACTH), α-melanocyte stimulating hormone (α-MSH), β-MSH and γ-MSH are derived from a 31- to 36-kDa precursor protein, proopiomelanocortin (POMC) [1]. The main site of their synthesis is the pituitary gland. α-MSH was isolated in 1955 from the pituitary extracts as one of the first peptide hormones. In the fifties, the amino acid sequence of ACTH and β-MSH were also reported. Analysis of the amino acid sequence of the isolated peptides has revealed that MSH is a part of ACTH and that α-MSH, β-MSH and ACTH share the same core sequence Met-Glu-His-Phe-Arg-Trp-Gly, moreover, β-MSH appeared to be a part of β-lipotropin (β-LPH). In the late seventies, the sequence of their precursor, proopiomelanocortin was established [2]. When the POMC gene was cloned, mRNA expression studies showed the existence of the brain melanocortin system, separate from the pituitary. In the rat brain, POMC expression is confined largely to the arcuate nucleus of the hypothalamus (Arc) and nucleus tractus solitarius (NTS) of the medulla. From these discrete sources, melanocortinergic fibers project widely throughout the brain. In general, the major site of POMC synthesis in the brain is the arcuate nucleus of the hypothalamus. Compared to the arcuate POMC neurons, the NTS cells contain much lower levels of POMC mRNA. The presence of POMC-derived peptides in central nervous system (CNS) pathways as well as in the pituitary gland, an integral component of the hypothalamo-pituituary-adrenal (HPA) axis, involved in stress-related phenomena, suggests that POMC may serve as a link between endogenous pain control and stress response systems in the body [3]. POMC contains also in its structure the amino acid sequences homological to the structures of a variety of peptides with different biological functions, which are generated via tissue-specific cleavage.

There is a growing body of evidence from many recent studies indicating that melanocortins and melanocortin receptors are crucially implicated in a variety of important functions, beside their effect on melanocytes. Melanocortins possess a large number of multifarious actions, inducing stimulatory action on learning, attention and memory, motor effects and inhibition of food intake. Finally, there is a strong evidence that among POMC-derived peptides, melanocortins are the ones that exert a variety of immunomodulatory and anti-inflammatory activities, and facilitate regeneration of injured nerves [4–6]. The latter functions have recently attracted much interest since melanocortins are the target of the search for better antinociceptive drugs in chronic pain, especially inflammatory and neuropathic pain. Moreover, they share a common precursor molecule, POMC, with opioid peptides, e.g. β-endorphin, and seem to counteract the opioid effects, like nociception, tolerance etc, which is of interest in coping with side effects of opioids. Therefore, in this paper we review the functions of melanocortins, particularly those which are connected with they role in inflammation, injury and pain.

Melanocortin system

POMC sequence comprises the sequence of the melanocortin peptides: ACTH, α-, β-, γ-MSH. POMC also contains one copy of the opioid-defining amino acid sequence, Tyr-Gly-Gly-Phe-Met; which is found at the NH_2-terminus of the opioid peptide, β-endorphin (β-End), and lipotropic hormone (LPH). Peptide hormones and transmitters are synthesized as high molecular weight precursors, prohormones, which are processed to yield smaller active fragments. The proteolytic processing of POMC occurs through cleavage of its molecule by the prohormone convertase PC1 and PC2, that

recognize the pairs of basic residues (lysine and arginine) and cleave the bond between them. This is followed by the removal of the carboxy-terminal basic residue by carboxy-peptidase E (CPE) [7,8]. Proconvertase 1 (PC1) generates ACTH and β-LPH, while PC2 generates α-MSH, by the cleavage of ACTH, and β-End. The entire 13 amino acid sequence of α-MSH is contained within the N-terminal region of ACTH, which is a 39 amino acid peptide. Further posttranslational modifications of α-MSH include amidation of the peptide at the C terminus and acetylation at its N terminus. All melanocortins share a conserved tetrapeptide sequence, Met-Glu-His-Phe-Arg-Trp-Gly, which is important for their effects [9].

α-MSH has been detected in many peripheral tissues, i.e. in the pituitary, circulation, kidney, intestine, adrenal glands, pancreas, ovaries, testis, placenta and skin, where it shows its paracrine action. α-MSH is prominent in the brain tissues and can also be found in the CNS structures, such as the arcuate nucleus of the hypothalamus and nucleus tractus solitarius of the brainstem [10]. The nerve fibers project from the above-listed structures to the hypothalamus, thalamus, mesencephalon, amygdala, hippocampus, cortex, medulla and spinal cord [10]. Cloning of five different melanocortin receptors (MC1R-MC5R) in the period between 1992–1994 started a new era in the research on the melanocortin receptors. Until that time, the receptors of melanocortins were mainly known for their physiological effects, such as the regulation of skin pigmentation by α-MSH and ACTH-induced secretion of corticosteroids. However, MSH/ACTH peptides were also known to induce an additional wide range of effects, including both central effects such as alterations in motor and sexual behavior, analgesia, improvement of memory, antipyretic effects and peripheral effects such as powerful anti-inflammatory and lipolytic action.

The melanocortin receptors

The first data on cloning of melanocortin receptors spurred melanocortins research. On the basis of homology with other members of the large family of G-protein coupled receptors, the mouse and human MSH (MC1) and ACTH (MC2) receptors were cloned from cDNA isolated from melanoma cells containing a high level of specific [^{125}I]NDP-α-MSH binding [11,12]. To date, five melanocortin receptor (MCR) subtypes have been cloned [11–15]. Each receptor is the product of a separate gene [9]. These discoveries facilitated research into the physiological roles of the five melanocortin receptors, and the compounds with selective actions at some of them became available. Melanocortin receptors are proteins with seven transmembrane domains positively coupled to G_s proteins and belonging to the G-protein coupled (GPC) receptor family. When compared with other GPC receptors, they have a short second extracellular loop, an intracellular carboxy-terminal domains and a short extracellular amino-terminal domain. The melanocortin receptors exhibit sequence homologies ranging between 40% to 60%. All melanocortin receptors have several N-glycosylation sites in their N-terminal domains. They also have conserved cysteine residues in their C-termini (a potential site for acetylation with fatty acid). All five forms of melanocortin receptors are functionally coupled to adenylate cyclase (AC) and mediate their effects primarily by activating the cAMP-dependent signaling pathway. However, some authors also indicate phosphoinositol pathway for the MC3 receptor [16] and the Jak/STAT pathway for the MC5 receptor [17]. The melanocortin receptors have recognition sites for protein kinase C (PKC), and some also for protein kinase A (PKA) [11] indicating that they may be regulated by phosphorylation.

ACTH, α-MSH, β-MSH are potent, high affinity agonists of all melanocortin receptors except for the MC2 receptor. The melanocortin MC2 receptor is the most selective one, recognizing only ACTH sequence. Selectivity of melanocortin receptors is summarized in Table 1.

Table 1
Selectivity of melanocortin receptors

Receptor	Agonists in increasing order of affinity	mRNA expression	Function
MC1	α-MSH = ACTH > β-MSH>γ-MSH	Melanocytes, Leydig cells, testis, corpus luteum, placenta, macrophages/monocytes, neutrophils, endothelial cells, fibroblasts, glioma cells and astrocytes. Also in: pituitary and periaqueductal gray (PAG)	Hair and skin pigmentation, immunomodulation and anti-inflammatory effects
MC2	ACTH	Adrenal cortex	Lipolytic activity
MC3	α-MSH = β-MSH = γ-MSH = ACTH	Brain: hypothalamus, medial habenula nucleus, lateral septal nucleus, ventral tegmental area. Peripheral tissues: placenta, duodenum, pancreas, stomach	Cardiovascular functions, thermoregulation, control of feeding behavior
MC4	α-MSH = ACTH> β-MSH ≫ γ-MSH	Brain: hypothalamus, olfactory cortex, septal region, hippocampus, superior colliculus, nucleus of the optic tract, brainstem and spinal cord. During ontogeny: brain, spinal cord, autonomic nervous system and adrenal medulla	Involvement in autonomic and neuroendocrine functions, regulation of food intake, weight homeostasis, hyperalgesia, pain, grooming behavior, stress
MC5	α-MSH>ACTH = β-MSH ≫ γ-MSH	Peripheral tissues: skin, adrenal gland, skeletal muscle, bone marrow, spleen, thymus, testis and ovary, uterus, lung, liver, thyroid, thymus, stomach, kidney, exocrine glands. Brain: cortex, cerebellum	Function not very well understood, and mostly speculative: neuro/myotropic, gastric and inflammatory effects, regulation of aldosterone secretion, weak lipolytic activity of α-MSH on adipocytes, regulation of hair lipid production, water repulsion, thermal regulation, exocrine gland function

Current knowledge still does not allow us to evaluate adequately the physiological importance of existence of melanocortin receptor subtypes and their ability to recognize MSH peptides. However, the cloning and characterization of melanocortin receptors capacitated relating the MC receptors to various biological actions of melanocortin peptides.

MC1 receptor

MC1 receptor was the first member of the melanocortin receptor gene family which was cloned. It was cloned from a mouse melanoma cell line and from a primary culture of normal human melanocytes by Mountjoy et al., [11] and from human melanoma cell by Chhajlani and Wikberg [12]. MC1 is the receptor for α-MSH on melanocytes. Among all cloned melanocortin receptors, MC1 receptor has the highest affinity for α-MSH [18]. α-MSH is the physiological regulator of rapid color change in lower vertebrate species, including fish, amphibians, and reptiles, and a stimulator of melanogenesis in mammalian melanocytes [19]. The MC1 receptor was also found on the Leydig cells of the testis, lutein cells of the corpus luteum, trophoblastic cells of the placenta, macrophages and monocytes [20,21], neutrophils [22], endothelial cells [21], fibroblasts [23], glioma cells and astrocytes [24]. Recently, it has also been discovered that out of the five cloned melanocortin receptors, monocytes express only the MC1 receptor. The MC1 receptor expression on monocytes is up-regulated by mitogens, endotoxins, and proinflammatory cytokines [25]. In situ hybridization studies and immunohistochemical methods demonstrated the

presence of MC1 receptor on a few scattered neurons of the periaqueductal grey matter in both rat and human brains [5]. Chhajlani [26] have also reported that the MC1R is present in the pituitary.

MC2 receptor

Shortly after cloning of MC1 receptor, MC2 receptor was cloned from the adrenal gland [11,15]. ACTH preferentially binds to MC2 receptor, hence it is considered to be the ACTH receptor. MC2 receptor is mainly expressed in the zona fasciculate (site of glucocorticoid production) and in zona glomelurosa (site for mineralocorticoid production) in the adrenal cortex [11]. MC2 receptor plays a role in mediating ACTH effect on steroid secretion [27]. The mRNA for ACTH (MC2) receptor and mRNAs for three enzymes obligatory for steroid synthesis including cytochromes P450scc, P450c17 and P450c21 were shown using RT-PCR method to be expressed in normal and pathologic human skin [28]. MC2R is also expressed in the white adipose tissue of rodents [23] but, in contrast, human adipocytes do not express melanocortin MC2 receptor [27]. MC2 receptor, expressed in adipocytes of various mammals mediates mostly lipolytic activity of ACTH [29].

MC3 receptor

The third type of melanocortin receptor is MC3 receptor. Human MC3 receptor is 361 amino acid long [13,30]. It has no specificity in recognition of individual melanocortins, and shows similar binding affinity for α-, β-, γ-MSH, and ACTH. MC3 is expressed in the periphery: placenta, stomach, duodenum, pancreas, gut [13] and in the heart [12]. It is also detectable in the testis, ovary, mammary gland, skeletal muscle and kidney [13,26]. MC3R is widely expressed within the CNS: in the hypothalamus, thalamus, hippocampus, anterior amygdala, and in the cortex. This distribution suggests its role in the regulation of cardiovascular functions and thermoregulation, as well as in the control of feeding behavior [31].

High densities of MC3 receptor are also present in the ventromedial nucleus of the hypothalamus (including the arcuate nucleus) and posterior hypothalamus. ^{125}I-NDP-MSH binding studies [32] revealed high densities of the MC3 receptor in the ventromedial nucleus of the hypothalamus and in the nucleus accumbens, and also in the medial preoptic area and central gray. The presence of MC3 receptor in the nucleus accumbens is of great interest in relation to the reported contribution of the melanocortin system to morphine addiction [33] and other forms of drug addiction. High levels of MC3 receptor are also detectable in the lateral septum and olfactory tubercle, also in the optic nerve layer of the superior colliculus [34].

MC4 receptor

Human MC4 receptor was the second neural melanocortin receptor which was cloned. It is 333 amino acid long [14]. Detailed studies covering many human organs failed to demonstrate the melanocortin MC4 receptor expression in the periphery [26]. However, the MC4 receptor expression is quite widespread in the brain, as it was detected in the thalamus, hypothalamus, brainstem, and cortex. MC4 receptor is also expressed in the spinal cord, superficial dorsal horn; lamina I, II and the gray matter surrounding the central canal: lamina X [35]. MC4 receptor distribution in the cortex, thalamus, hypothalamus and brainstem suggests its involvement in autonomic and neurocrine functions. The distribution of MC4 receptor in the CNS is much wider than the expression of MC3 receptor. The MC4 receptor is abundant in the paraventricular nucleus, which suggests its role in the central control of pituitary function. However, MC3 receptor may still have a dominating role compared to the MC4 in the medial part of the brain [32].

MC5 receptor

The MC5 receptor gene was the last cloned melanocortin receptor gene [36]. This receptor shows the highest sequence homology to MC4 receptor and the lowest homology to MC2 receptor. MC5 receptor mRNA is expressed at high levels in the exorcine glands, such as lacrimal and Harderian glands [37]. It is also expressed in skin tissues (i.e. sebaceous gland) and in the skeletal muscles. The MC5 receptor is expressed in many peripheral tissues. Its expression has been shown by Northern blot analysis, RT-PCR or RNase protection assay in the adrenal glands, fat cells, kidneys, liver, lung, bone marrow, thymus, mammary glands, testis, ovary, uterus, pituitary, stomach, skin, and skeletal muscles [26,38,39]. Although in situ hybridization in the brain proved to be unsuccessful, RT-PCR studies indicate that MC5 receptor is present in several brain regions, including the olfactory bulb, substantia nigra and striatum [40]. Expression of MC5 receptor mRNA has been detected in mouse adipocytes but at a lower level than MC2 receptor mRNA. The functions of MC5 receptor are still not very well understood. MC5 receptor might mediate the weak lipolytic activity of α-MSH on the adipocytes of several rodent species [23] and regulate hair lipid production, water repulsion and thermal regulation [37,41]. MC5 receptor is a prime candidate for mediating the secretion of stress-induced alarm substances, or stress pheromones [41].

Synthetic ligands of melanocortin receptors

The cloning of melanocortin receptors opened the way for identification of melanocortin receptor antagonists and other synthetic compounds displaying affinity for MC receptors. Major progress was made with the development of synthetic α-MSH analogues. One of the most useful synthetic peptides showing agonistic properties is melanotan-I, (Nle^4, D-Phe^7)α-MSH (NDP-MSH). NDP-MSH is the most potent linear MSH analogue [42]. Norleucine present in its structure prevents it from inactivation by oxidation. NDP-MSH shows high affinity for the melanocortin receptors with the following order of potency: MC1R>MC3R>MC4R>MC5R. The studies of the structure of α-MSH led to the discovery of a small, cyclic peptide, Ac-Nle^4-cyclo(5β→10∈)(Asp^5-His^6-D-Phe^7-Arg^8-Trp^9-Lys^{10})-amine^2 (MTII) [43]. Melanotan-II (MTII) action at melanocortin receptors is quite potent, but it is not particularly selective towards the particular receptor type [44], MTII has also been reported to be a potent but not selective agonist of the human MC3, MC4 and MC5 receptors [43]. Encompassed by the lactam ring of MTII, the 6–9 fragment of α-MSH, His^6-Phe^7-Arg^8-Trp^9, is regarded as an ''active site'' essential for the interaction of melanocortins with their receptors. It is well established that the N-terminal segment of the MTII (Ac-Nle^4, not included in the lactam ring) is one of the principal structural features determining potency and selectivity of MTII at the human melanocortin MC3-MC5 receptors. The Ac-Nle^4 fragment seems to have no effect on binding affinity and activation of the human MC4 receptor but it appears to be indispensable for the full potency at the human MC5 receptor [45]. Later, in 1995 Abou-Mohamed and his colleagues [46] reported a linear MSH peptide analogue, HP-228. An agonist of melanocortin receptors, HP-228, shows the following order of affinity for the melanocortin receptors: MC1R>MC3R>MC4R>MC5R.

The group of melanocortin receptor antagonists is represented by SHU9119 and HS014. Substitution of Phe^7 with β-(2-naphthyl)-D-alanine in MTII yielded SHU9119, the first antagonist of MC3 and MC4 receptors [47]. SHU9119 (Ac-cyclo-(Nle^4-Asp^5-D-Nal(2)^7-Lys^{10})α-MSH$_{4-10}$-NH$_2$) is a cyclic lactam peptide, which shows selectivity and antagonist activity at the melanocortin receptors. Attempts to iodinate Phe^7, hydrophobic amino acid in the α-MSH peptide, converted the peptide from an agonist to an antagonist of the MC3 and MC4 receptor. SHU9119 was found to be a potent, non-selective antagonist of MC3 and MC4 receptors.

Wikberg and coworkers [5] supplemented the series of cyclic MSH analogues by another selective MC4 receptor antagonist, HS014, the first selective MC4 receptor ligand. Its selectivity for this receptor is about 20-, 30- and 200-fold higher than for MC3, MC1 and MC5 receptors, respectively. HS014 is also an antagonist of MC3 receptor and a partial agonists of MC1 and MC5 receptors [48]. SHU9119 and HS014 are widely used to elucidate the physiological mechanisms underlying various effects of MSH peptides and their receptors, in particular to study in vivo pharmacology of MC4 receptor. SHU9119 has been used in some important initial studies into the roles of the neural MC3 and MC4 receptors [49,50], while HS014 was shown to increase the food intake of freely feeding rats [51,52]. HS014 has about 20-fold higher affinity for the MC4 receptor than for the MC3 receptor in binding assays, whereas SHU9119 binds with similar potency to both receptors. Both, HS014 and SHU9119 are partial agonists of MC1 and MC5 receptors. New data imply that the C-terminal part of HS014/β-MSH (Pro^{13}-Lys^{14}-Asp^{15}), in particular the Lys^{14} is indeed very important for its affinity for MC4 receptor without having any particular influence on the affinity for other MC receptors. In 1998 Kask and coworkers reported [53] another potent selective antagonist of MC4 receptor designated as HS024. The affinity of HS024 for MC4R is in the sub-nanomolar range. HS024 was shown to be an antagonist of MC3 and MC4 receptors, similarly as it was reported for SHU9119 and HS014. However, in contrast to these peptides, HS024 is also an antagonist of MC1 and MC5 receptors. HS024 did not show agonistic activity at any of melanocortin MC1, MC3, MC4 or MC5 receptors. In recent years, a new series of cyclic MSH analogues were designed and synthesized [48,53]. HS963, HS964, HS005 and HS006 are all [Cys^4-X^7-Cys^{11}]αMSH_{4-11} analogues and differ only in position 7. HS007, HS009 and HS011 are based on HS964 structure and differ in position 5, 6 and 10. Further expansion of the ring size was attempted in HS010 where Nle^4 was added to the ring. Two other peptides were constructed, which have

Table 2
Structure of some novel α-MSH analogues aligned with the original α-MSH sequence

Peptide	Structure	Reference
α-MSH	Ac-Ser^1-Tyr^2-Ser^3-Met^4-Glu^5-His^6-Phe^7-Arg^8-Trp^9-Gly^{10}-Lys^{11}-Pro^{12}-Val^{13}-NH_2	
NDP-α-MSH	Ac-Ser^1-Tyr^2-Ser^3-Nle^4-Glu^5-His^6-D-Phe^7-Arg^8-Trp^9-Gly^{10}-Lys^{11}-Pro^{12}-Val^{13}-NH_2	[54]
MTII	Ac-Nle^4-c(Asp^5-His^6-D-Phe^7-Arg^8-Trp^9-Lys^{10})-NH_2	[44]
SHU9119	Ac-Nle^4-c(Asp^5-His^6-**D-Nal^7**-Arg^8-Trp^9-Lys^{10})-NH_2	[47]
HS964	Ac-Cys^4-Glu^5-His^6-**D-Nal^7**-Arg^8-Trp^9-Gly^{10}-Cys^{11}-NH_2	[48]
HS963	Ac-Cys^4-Glu^5-His^6-**D-Phe^7**-Arg^8-Trp^9-Gly^{10}-Cys^{11}-NH_2	[48]
HS005	Ac-Cys^4-Glu^5-His^6-**D-Cha^7**-Arg^8-Trp^9-Gly^{10}-Cys^{11}-NH_2	[48]
HS006	Ac-Cys^4-Glu^5-His^6-**D-Bpa^7**-Arg^8-Trp^9-Gly^{10}-Cys^{11}-NH_2	[48]
HS007	Ac-Cys^4-**Arg^5**-His^6-D-Nal^7-Arg^8-Trp^9-Gly^{10}-Cys^{11}-NH_2	[48]
HS009	Ac-Cys^4-Glu^5-**His^6**-D-Nal^7-Arg^8-Trp^9-Gly^{10}-Cys^{11}-NH_2	[48]
HS011	Ac-Cys^4-Glu^5-Ala^6-D-Nal^7-Arg^8-Trp^9-**Asp^{10}**-Cys^{11}-NH_2	[48]
HS010	Ac-**Cys^3**-Nle^4-Glu^5-His^6-D-Nal^7-Arg^8-Trp^9-Gly^{10}-Cys^{11}-NH_2	[48]
HS012	Ac-**Nle^3**-Cys^4-Glu^5-His^6-D-Nal^7-Arg^8-Trp^9-Gly^{10}-Cys^{11}-NH_2	[49]
HS014	Ac-Cys^4-Glu^5-His^6-D-Nal^7-Arg^8-Trp^9-Gly^{10}-Cys^{11}-**Pro^{12}-Pro^{13}-Lys^{14}-Asp^{15}**-NH_2	[48]
HS024	Ac-Cys^3-Nle^4-Arg^5-His^6-D-Nal^7-Arg^8-Trp^9-Gly^{10}-Cys^{11}-NH_2	[53]

In bold letters of the substitutive amino acid sequence are given (HS963, HS964, HS005 and HS006 are all [Cys^4-X^7-Cys^{11}]αMSH_{4-11} analogues and differ only in position 7; HS007, HS009 and HS011 are based on HS964 structure and differ in position 5, 6 and 10; Expansion of the ring size (Nle^4 added) yielded HS010; Peptides with either C- or N-terminal extensions to the core cyclic structure of HS964: HS012-Nle^3 at the N-terminal end and HS014 has a 4-amino acid C-terminus which is identical to that of β-MSH.

either C- or N-terminal extensions to the core cyclic structure of HS964: HS012 has Nle^3 at the N-terminal end and HS014 has a 4-amino acid C-terminus which is identical to that of β-MSH. The structures of novel substances aligned with the original α-MSH sequence are summarized in Table 2. The 26-membered cyclic [Cys^4, D-Nal^7, Cys^{11}]αMSH_{4-11}, an analogue of HS964 has more than 12-fold MC4/ MC3 receptor selectivity and more than 60-fold MC4/ MC1 receptor selectivity. The ring size and the D-Nal^7 is crucial to obtain selective properties. His^6 together with Phe^7-Arg^8-Trp^9 make up the central core binding sequence of the MSH peptides. However, His^6 is probably less important than Phe^7, Arg^8 and Trp^9 for the binding of α-MSH [48].

Functions of melanocortin peptides

Immune system modulation

POMC peptides are produced by many different cells and tissues including the immune system and skin. All major constituents of the epidermis such as keratinocytes, Langerhans cells and melanocytes were found to express POMC mRNA and to release POMC-derived peptides [55]. Growing number of publications indicate that MSH peptides have a board capacity to inhibit inflammatory processes, viz. α-MSH was shown inhibit the inflammation in experimental bowel disease [56,57], arthritis, brain inflammation, brain ischemia [58–60], kidney ischemia [61], contact hypersensitivity and dermatitis [55]. α-MSH level is increased in inflammatory diseases, presumably as a natural countermeasure to inflammation [62]. The possibility to use drugs active at melanocortin receptor in the treatment of inflammatory processes has recently become a subject of many studies.

Antipyretic effect

Fever is the result of a shift in a balance between pyrogenic and cryogenic cytokines and hormones. Although there is considerable evidence that fever evolved as a host defense response, it is important that the rise in body temperature is not too high. Many endogenous cryogens or antipyretics that limit the rise in body temperature have been identified during the last 25 years. Endogenous mediators of fever include cytokines, among which interleukin-1 (IL-1) and IL-6 are considered the most important [63]. Studies of Kozak [64] and coworkers, have revealed that, besides pyrogenic cytokines, there are also cytokines that can be defined as endogenous antipyretics, such as tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-10 [64]. AVP, α-MSH and glucocorticoids can act as endogenous antipyretics counteracting the effects of pyrogens. α-MSH is one of the most potent endogenous antipyretic agents [65–69]. During fever, the α-MSH concentrations are increased in the septal region of the brain [70,71], suggesting that the endogenous α-MSH has a physiological role in fever control. The antipyretic effect of melanocortins is not mediated by the adrenal glands, since inhibition of fever by α-MSH is also observed after adrenalectomy [67]. In vivo studies have revealed that the melanocortin receptor antagonist, SHU9119, administered intracerebroventricularly (i.c.v.) worsens endotoxin-induced fever in rats [72]. In rats treated with lipopolysaccharide (LPS), i.c.v. injection of SHU9119 significantly increased fever, whereas its intravenous (i.v.) injection at the same dose had no effect. Neither i.c.v. nor i.v. SHU9119 treatment significantly affected LPS-stimulated plasma ACTH or corticosterone levels. Thus, the results indicate that endogenous central melanocortins exert an antipyretic

influence during fever by acting on melanocortin receptors located within the brain, independently of any modulation of the activity of the pituitary-adrenal axis.

The melanocortin MC3 and MC4 receptors are expressed in the brain regions involved in thermoregulation, such as the septum, preoptic area, anterior hypothalamus [35], further indicating that these receptors are important for the regulation of body temperature during fever. Peripheral injections of α-MSH also induce antipyretic action, although the fever reduction is weaker when compared with the effect of the centrally administered α-MSH.

The centrally administered α-MSH is a potent antipyretic peptide in different species [67]. I.c.v. injections of low doses of α-MSH inhibit fever induced by endotoxins or cytokines, such as IL-1, IL-6 and TNF-α [67]. Linear MSH analogue, NDP-MSH is even more potent in reducing fever than centrally administered α-MSH. I.c.v. doses of α-MSH (30 and 300 ng) potentiated the suppressive effects of LPS on food intake and locomotion, despite the fact that the higher dose alleviated LPS-induced fever. The above results suggest that exogenous and endogenous melanocortins acting centrally divergently influence different aspects of the acute phase response, suppressing LPS-induced fever but contributing to LPS-induced anorexia and hypoactivity [73]. The non-selective melanocortinergic peptides, MTII and SHU9119 are used in current research to investigate the role of melanocortin receptors in α-MSH-mediated fever alleviation. MTII (1 mg/kg, i.p.), an MC1, MC3, MC4 and MC5 receptor agonist, inhibited LPS (20 μg/kg, i.p.)-induced hyperthermic response. In contrast, SHU9119, an MC1 and MC5 receptor agonist and MC3 and MC4 receptor antagonist, had no effect on this response [74]. Thus, it seems that only MC4 receptor might play a role in α-MSH-induced antipyretic action.

Anti-inflammatory effect

The melanocortin peptides have been effective in major models of inflammation, and more recent tests have been extended to include CNS ischemia, reperfusion injury and bacterial endotoxin-induced inflammation within the brain [67]. They have been reported to inhibit different types of inflammatory responses including acute and chronic inflammation and contact hypersensitivity [75]. Centrally (i.c.v.) administered α-MSH inhibits the TNF-α response in endotoxin-induced brain inflammation [58].

Interestingly, centrally injected α-MSH also reduces inflammation in peripheral tissues. Lotti et al. [76] reported that the α-MSH, as well as its C-terminal tripeptide sequence administered into the CNS inhibited locally induced cutaneous inflammation. Studies performed on mice have shown that i.c.v. administration of $\alpha\text{-MSH}_{1-13}$ and $\alpha\text{-MSH}_{11-13}$ inhibits cutaneous inflammation induced by application of IL-1β, IL-8 or platelet activating factor, probably through descending neurogenic pathways capable of modulating inflammation in peripheral tissues [76]. The inhibitory influence of centrally administered α-MSH probably involves the neuron-mediated release of local neurogenic components of inflammation, such as vasointestinal peptide (VIP) or calcitonin gene related peptide (CGRP). The precise mechanisms of action of α-MSH peptides are still uncertain, although neural pathway integrity is necessary to preserve the anti-inflammatory action of centrally administered α-MSH. Systemic injection of α-MSH in animals with spinal cord transsection resulted in much weaker anti-inflammatory effect. However, $\alpha\text{-MSH}_{11-13}$ injected intraperitoneally (i.p.) exerts its anti-inflammatory effect even after the cutting of the spinal cord in mice, indicating a more marked anti-inflammatory activity of the C-terminal tripeptide in the periphery [76,77].

Peripheral α-MSH also displays anti-inflammatory effects. α-MSH was detected in many peripheral tissues including skin. Peripheral expression of POMC gene has been detected in the rat peritoneal macrophages and the POMC gene products seem to be processed to produce immunoreactive ACTH.

Moreover, peritoneal macrophages express MC3 receptor mRNA [78]. Getting et al. [78] reported that peptides containing $ACTH_{4-10}$ sequence suppressed peritoneal macrophage accumulation in acute inflammation. The above data identify MC3 receptor as the molecular target for these peptides. MC3 receptors may operate to down-regulate the acute inflammatory response or the acute phases of chronic inflammation. The effects of the natural and synthetic ligands of the MC3 receptor have also been evaluated in a murine model of experimental gout. Naturally occurring melanocortins, as well as the synthetic long-acting compound MTII, activate MC3 receptor on peritoneal macrophages to inhibit the experimental inflammatory response [79].

In monocytes, macrophages and dendritic cells, α-MSH inhibits the production and activity of immunoregulatory and proinflammatory cytokines, such as IL-2, interferon γ (IFNγ) and IL-1. It down-regulates the expression of co-stimulatory molecules, such as CD86 and CD40, and induces the production of suppressor factors, such as the cytokine synthesis inhibitory factor IL-10 [75]. There is evidence that autocrine circuits, based on α-MSH and its receptors, may operate in both murine and human monocyte/macrophages that modulate the production of inflammatory agents, thereby limiting local inflammatory processes. In murine macrophages, α-MSH inhibits inflammation-related nitric oxide (NO) production by inhibiting the expression of inducible NO synthase (iNOS) [20]. These cells contain MC1 receptor mRNA, and they secrete α-MSH, when stimulated with LPS or IFNγ with the addiction of TNF-α. The cells of THP-1 human monocyte/macrophage cell line produce little NO but secrete neopterin, a marker of macrophage activation. α-MSH inhibits neopterin production induced by co-culture of THP-1 cells with IFNγ and TNF-α. These observations raise the hypothesis that an endogenous autocrine anti-inflammatory circuit exists in macrophages of human origin that depends on the neuropeptide α-MSH and specific melanocortin receptors [80]. Furthermore, the α-MSH inhibits neutrophil chemotaxis in vitro [70]. Prevention of neutrophil migration results in moderation of inflammation. Both, neutrophils and monocytes express melanocortin receptors, and it is clear that α-MSH has direct effects on these inflammatory cells.

Recently it has also been shown that melanocortins are implicated in sepsis, which is an inflammatory disorder difficult to understand and to treat. Despite the recent upsurge of information about inflammatory mediators in sepsis and promising therapeutic leads (e.g. anti-TNF-α antibodies, IL-1 receptor antagonist), over the last 30 years there has been little increase in survival rate in sepsis patients, and there is no agreement about medical therapy for sepsis [81]. In recent experimental studies, mice given a systemic injection of LPS and central injection of saline died within 48 hours, whereas almost 50% of animals given a single, central injection of α-MSH survived and were alive 3 month after the neuropeptide injection [82]. This suggests that the prevention or reversal of disturbances within the brain, like the changes perhaps marked by an increase in local production of TNF-α and related proinflammatory agents, plays a key role in control of sepsis. Targeting of CNS melanocortin receptors may lead to successful treatment of sepsis [82].

A number of studies suggest that α-MSH inhibits production of proinflammatory cytokines via the modulation of nuclear transcription factor-κB (NF-κB) activation [60]. Activation of NF-κB may be elicited by a large number of stimuli which include bacterial and viral infections, oxidative stress, LPS, IFNγ and pro-inflammatory cytokines (IL-1, TNF-α) [83–85]. α-MSH appears to function as a general inhibitor of NF-κB activation thereby exerting its anti-inflammatory and immunomodulatory effects [82]. NF-κB participates in the regulation of over 150 target genes, which include growth factors of hematopoetic system, cytokines, chemokines, major histocompatibility complexes, antiapoptotic factors and inducible nitric oxide synthase [84,86]. Activation of NF-κB requires degradation of the

cytoplasmatic inhibitor IκBα protein. α-MSH inhibits nuclear translocation of NF-κB by slowing degradation of IκBα induced by LPS in astrocytoma cells [87]. Similar effect was noticed in the brain tissue when α-MSH was injected centrally after i.c.v. administration of LPS. Chloramphenicol acetyltransferase (ChAT) assay indicated that α-MSH suppressed NF-κB-dependent reporter gene expression induced by LPS in glioma cells [88]. The above findings suggest that the anti-inflammatory action of α-MSH occurs via inhibition of production of inflammatory agents by modulation of NF-κB activation both in peripheral and central tissues [88]. A number of studies suggest that the inhibition of NF-κB is a focal point in the mediation of the effects of melanocortins on cells of the immune system. α-MSH antagonizes the proinflammatory activities of cytokines [55], and inhibits the IL-1-induced activation of NF-κB. Therefore, NF-κB inactivation by α-MSH appears to be a crucial event, and it can be responsible for α-MSH-induced down-regulation of cytokine gene transcription [86].

Melanocortins have also been shown to affect many cell types in a way that suggests that they may contribute to the down-regulation of immune response. In endothelial cells, α-MSH is capable of down-regulating LPS-induced expression of adhesion molecules, such as vascular cellular adhesion molecules (VCAM) and E-selectins, which was demonstrated on a human dermal microvascular endothelial cell (HDMEC) line [89]. Reduction of adhesion molecules on the cell surface may contribute to an anti-inflammatory action by a diminished recruitment of inflammatory cells. Endothelial cells play a crucial role in inflammation and immune system reactions. Upon stimulation, both endothelial cells and inflammatory cells express vascular adhesion molecule, intercellular adhesion molecule (ICAM), integrins and E-selectins [90]. Proinflammatory agents: IL-1, LPS or TNF-α up regulate the expression of adhesion molecules, allowing the inflammatory cells to adhere and migrate into the tissues surrounding the inflamed region. Therefore, the down-regulation of endothelial cell adhesion molecule expression appears to be an important event in the control of inflammation. The capacity of α-MSH to modulate the expression of adhesion molecules was investigated on HDMECs along with melanocortin receptor expression on these cells. Under normal conditions, HDMECs express low level of MC1 receptor, that is significantly up regulated upon stimulation by proinflammatory cytokines, such as IL-1 and α-MSH itself [21]. Moreover, Kalden and his colleagues [89] reported that α-MSH in a dose-dependent manner was capable of down-regulating the LPS-induced expression of mRNA coding for ICAM-1, VCAM and E-selectin and also protein level [89]. This observation was further confirmed by in vitro studies demonstrating that α-MSH significantly suppresses LPS-mediated adhesion of lymphocytes to monolayer of HDMECs [89]. These findings indicate that α-MSH via modulating the expression of different adhesion molecules is capable of preventing adhesion and transmigration of inflammatory cells and thus contributes to the down-regulation of an inflammatory response [82].

Skin is a target organ and a peripheral source of POMC [91]. POMC-derived peptides detected in skin are expressed by epidermal and dermal cells such as melanocytes, keratinocytes and fibroblasts as well as by inflammatory cells including cutaneous monocytes, macrophages and neutrophils [55,91]. Transcription and release of skin POMC peptides in vivo and in vitro is increased after trauma, infection [92], or exposure to ultraviolet (UV) light. Among POMC peptides, α-MSH is regarded as a neurohormone with extensive immunomodulatory capacities [25]. Significant role of MC1 receptor and its ligands was examined by Scholzen et al. [93], who showed that HDMECs synthesized POMC mRNA and released the POMC peptides: ACTH and α-MSH, which could be regulated by IL-1 or UV light. The authors suggest that UV light is capable of up-regulating the expression of POMC in HDMECs and also of elevating the level of PC1, one of the major proteolytic enzymes required to process POMC prohormone. Thus, these data [93] provide evidence that HDMEC are capable of

expressing functional MC1 receptor, POMC, and PC1 mRNA; and of releasing POMC peptides with UV light, IL-1, and α-MSH as regulatory factors. The expression and regulation of these peptides may be of importance, not only for the autocrine or paracrine regulation of physiologic functions of dermal endothelial cells, but also for the regulation of certain microvascular-mediated cutaneous or systemic inflammatory responses.

The MC1 receptor expression in sebocytes of normal human skin was further confirmed by immunohistochemical studies. Modulatory action of α-MSH on IL-8 release by IL-1β-stimulated sebocytes was demonstrated by Bohm et al. [94]. Sebocytes secrete IL-8 in a constitutive manner. IL-8 is a chemokine that attracts inflammatory cells to the sebaceous gland and initiates inflammatory response. The authors have demonstrated that sebocytes express MC1 receptor in vitro and in situ and that α-MSH can partially abrogate the inductive effects of IL-1β on IL-8 secretion by sebocytes. Thus, by modulating IL-8 secretion, α-MSH may act as a modulator of inflammatory responses in the pilosebaceous unit.

The presence of α-MSH in gut and skin suggests that this potent anti-inflammatory molecule may be a component of the innate host defense. α-MSH showed inhibitory influences on the gram-positive bacterium *Staphylococcus aureus* and the yeast *Candida albicans* [95]. The active message sequence resides in the C-terminal tripeptide $\alpha\text{-MSH}_{11-13}$ (KPV), which has anti-inflammatory influences in vivo and in vitro that parallel those of the parent molecule [96,97]. α-MSH immunoreactivity has also been found in the mucosal barrier of the gastrointestinal tract, further confirming its role in host defense. The antimicrobial influences of α-MSH are reviewed in Catania et al. [95]. In short, in experiments on *Staphylococcus aureus*, the authors demonstrated that α-MSH and its C-terminal tripeptide KPV, used in a wide concentration range, inhibited *Staphylococcus aureus* and *Candida albicans* colony formation [95,98]. α-MSH reduced not only *Candida albicans* viability, but also germ tube formation, which is associated with increased virulence of the yeast [95]. Many of the α-MSH effects are mediated by cAMP [10]. It has been demonstrated that the agents enhancing cAMP level inhibit mRNA and protein synthesis in *Candida albicans* [99]. Therefore, it is likely that the antimicrobial effect of α-MSH is caused by enhanced production of this mediator.

Replication of human immunodeficiency virus (HIV) is dependent on the state of activation of the infected immune cells and is regulated by interactions between viral and host factors [100]. Proinflammatory cytokines have a prominent enhancing effect on HIV replication [101]. TNF-α, IL-1 and IL-6 promote HIV replication and influence the disease progression [102]. The capability of α-MSH to counteract the actions of tumor necrosis factor and its antimicrobial effects suggest that the peptide might likewise reduce replication of HIV. Indeed, the treatment with α-MSH reduced HIV replication in chronically and acutely infected human monocytes. At the molecular level, α-MSH inhibited activation of the transcription factor NF-κB known to enhance HIV expression. α-MSH that combines antipyretic, anti-inflammatory, and antimicrobial effects could be useful in the treatment of disorders in which infection and inflammation coexist [95].

The potent and broad anti-inflammatory actions of α-MSH, coupled with its extremely low toxicity and its effectiveness when administered centrally, raise the possibility that it might be useful in the treatment of inflammatory and neurodegenerative brain disorders [62]. TNF-α underlies pathological processes and functional disturbances in acute and chronic neurological diseases and nervous tissue injury. TNF-α occurs in abundance in lesion sites in multiple sclerosis (MS) and other neurodegenerative disorders such as Alzheimer's disease. The capacity of TNF-α to promote myelin destruction and to increase expression of adhesion molecules makes it a prime suspect in the etiology of MS lesions [62].

The studies of Rajora and coworkers [58] demonstrated the inhibitory effect of α-MSH on the brain TNF-α. The presence of mRNA for the α-MSH receptor MC1 within the normal mouse brain suggests that the inhibitory effects of α-MSH on the brain and plasma TNF-α might be mediated by this receptor type. Since central TNF-α contributes to the pathological changes during the neurodegenerative brain diseases and CNS injury, and promotes inflammation in the periphery, the agents that act on the brain α-MSH receptors should decrease the damaging TNF-α effects and promote tissue survival [58].

Summing up, many of the cell types responsive to melanocortins have been reported to bear MC1 receptor, e.g. macrophages and monocytes, neutrophils, fibroblasts, endothelial cells, glioma cells, melanocytes and melanoma cells. It has been demonstrated that normal human monocytes contain low number of MC1 receptor binding sites which become up-regulated upon activation of the monocytes by various agents, such as LPS, or combination of cytokines [103]. Thus, the MC1 receptor seems to be a strong candidate for the mediator of the effects of the MSH-peptides on these cells. However, Getting et al. [78] have presented evidence for the expression of the MC3 receptors in murine macrophages. Moreover, Buggy et al. [17] confirmed the presence of the MC5 receptors on the B-lymphocyte cell line. Artuc et al. [104] demonstrated the presence of both MC1 and MC5 receptors on a human mast cell line. Thus, these findings open the possibility that several melanocortin receptors could participate in mediating the observed effects of MSH peptides. The above results, combined with prior evidence of effectiveness of α-MSH analogues in modulating inflammatory processes, and their low toxicity, suggest that these molecules may be useful in the treatment of CNS disorders that have an inflammatory component [24].

Nerve regeneration

Melanocortin peptides (α-MSH, ACTH) improve axonal regeneration following peripheral nerve injury and stimulate neurite outgrowth in the CNS neurons in vitro and in vivo. Following peripheral nerve injury or pathology, melanocortins are effective growth factors, accelerating and enhancing nerve regeneration and muscle reinnervation [6]. Melanocortins facilitate recovery of both sensory and motor functions following crush or transection of the sciatic nerve in rats [105].

Structure-activity relationship studies show that the neurotrophic effects of melanocortins are determined by the melanocortin core sequence of the ACTH/MSH molecule. The beneficial effects of MSH are dose-dependent and occur in a time interval restricted to a short period after nerve injury [105]. Both systemic and local application of α-MSH is effective in stimulating nerve regeneration, however, melanocortins stimulate the initial sprouting response in the damaged nerve, while the outgrowth rate of the sprouts is unaffected [106,107].

Adan and his colleagues [108] reported that α-MSH was able to stimulate neurite outgrowth in the neuroblastoma cell line Neuro2A. Moreover, the MC4 receptor antagonist (D-Arg^{8} $ACTH_{4-10}$) inhibited the stimulation of neurite outgrowth by α-MSH. α-MSH stimulates neurite outgrowth in cultures of the spinal cord and dorsal root ganglion (DRG) neurons [109] as shown by in vitro experiments. There are also literature data indicating that MSH-like peptides influence the growth and differentiation of central neurons in culture. Cerebral chicken neurons treated with $ACTH_{1-24}$ enhance the cell metabolism and increase neurite formation [110]. In rat embryonic cerebral cells in culture, the treatment with $ACTH_{4-10}$ and $ACTH_{1-24}$ resulted in an increase in the density of the neuronal network and in neurite bundles [111]. ACTH and its analogues promote the regeneration of crushed peripheral nerves as demonstrated by the accelerated return of motor and sensory functions [112]. Early axonal sprouting and improvement of the regeneration of motor units and formation of the complex reinnervation sites indicate a positive

role of the melanocortins [113]. This findings are supported by an increase in $ACTH_{4-10}$ levels in the motoneurons in both ventral horns, following unilateral peripheral nerve crush [114]. Damaged central neurons also appear to be functionally improved by melanocortin administration, most probably through neuroprotective action since these peptides are most beneficial when administered immediately after nerve damage [115–118].

MSH-like peptides play a beneficial role in the repair and development of the nervous system. Therefore, melanocortins can be considered as growth factors. They facilitate the recovery following the sciatic nerve crush in rats. The most active peptides are α-MSH and $ACTH_{4-9}$ analogue ORG2766. The neurotrophic influence of ACTH/MSH-like peptides was evident both at the sensory and the motor function level [119].

α-MSH is able to stimulate in vivo the spinal neurite outgrowth after the spinal cord trauma. Joosten and collaborators [120] reported that local applications of α-MSH stimulated the ingrowth of neurites into a collagen matrix that spans the lesion gap in rats with spinal cord lesions at the thoracic spinal level. A profound and significant stimulation of fiber ingrowth into the collagen implant in the lesion gap was observed. Moreover, α-MSH enhanced functional recovery in rats with the spinal cord lesions. The changes in the growth-associated protein B-50 have been suggested as one of the mechanisms underlying these effects of α-MSH [121].

The ability of exogenous melanocortins to stimulate peripheral nerve repair may reflect possible involvement of similar endogenous peptides in the physiological regenerative response following nerve injury. Although endogenous melanocortins are synthesized in the pituitary, it has been suggested that ACTH/MSH-like peptides are also formed in traumatized nerves in response to nerve injury. Biologically active MSH-like peptides have been detected in the extracts of degenerating nerves, whereas no activity was demonstrated in the extracts of control nerves [106]. α-MSH-like peptides may be formed in the degenerating distal nerve stump by specific proteolysis of the 150 kDa neurofilament protein or may derive from reexpression of POMC mRNA in the cell bodies of the damaged neurons. Other authors claim that endogenous neurotrophic melanocortins are derived from extra-pituitary expression of the POMC gene [122]. POMC-derived immunoreactivity has been described in the rat spinal cord, dorsal root ganglion and sciatic nerve [122], however, regulation of POMC gene could be demonstrated neither in the spinal cord or dorsal root ganglia nor in the sciatic nerve after the sciatic nerve crush in rats. No α-MSH could also be detected with radioimmunoassay method in the damaged rat peripheral nerves [123]. The mechanisms underlying the neurotrophic effects of α-MSH are not understood. The ability of α-MSH to stimulate neurite outgrowth in the cultured neurons indicates that α-MSH directly affects neurons, although glial cells also respond to α-MSH and NDP-MSH with a rise in cAMP levels, indicating that glial cells can be a target for melanocortins as well [124]. In vitro studies demonstrate that cAMP production is increased in neuronal cultures upon melanocortin treatment [124]. cAMP analogues stimulate neurite outgrowth in neural cultures [125] and enhance functional recovery after peripheral nerve crush in rats, emphasizing the importance of this second messenger in neurotrophic processes. Cyclic AMP and intracellular calcium both play an important role in regeneration. An increase in cAMP-induced neurite outgrowth by treatment with the cAMP analogue dbcAMP or the adenylate cyclase activator forskolin as was shown in vivo [126,127] and in vitro [128,129]. However, these data are in contrast to those published by Mattson and his colleagues [130] who have shown that an increase in cAMP level inhibits neurite outgrowth. Besides cAMP, intercellular calcium is also strongly involved in the regulation of neurite outgrowth [130,131], with narrow optimal range of $[Ca^{2+}]_i$ [132]. The melanocortin receptors are coupled to AC and PLC [16], thus are suitable candidates for stimulating

nerve regeneration and neurite outgrowth via cAMP and IP_3/calcium pathway [16]. It is likely that more than one melanocortin receptor type is involved in eliciting a specific response. The candidates to play a role in α-MSH-enhanced nerve regeneration are the three neural melanocortin receptors: MC3, MC4 and MC5, as they are expressed in the nervous tissue. Moreover, they all recognize the small peptide fragment, $ACTH_{4-10}$ that causes regeneration in vivo [133] and neurite outgrowth in vitro [109]. The expression of MC4 receptor in the spinal cord and MC5 receptor in the mouse skeletal muscles also indicates that these two receptors could play a role in the neurotrophic effect of α-MSH.

The spite of many studies, the exact nature of melanocortins as endogenous neurotrophic factors remains obscure.

Nociception

Recent data suggest that, in addition to their other diverse biological functions, melanocortin peptides might play a crucial role in nociceptive information processing and in alleviating pain symptoms. Melanocortins are known to have direct effects on nociception. In 1981 Sandman and Kastin [134] reported that i.c.v. injections of α-MSH produced hyperalgesia measured by tail-flick test over the first 20 min, or hyperalgesia throughout the 80 min of the testing, depending of the dose. Delivery of α-MSH peptide into the brain ventricles modulates the threshold of sensitivity to pain and produces a dose-dependent analgesia, which can be blocked by a 1 mg/kg subcuteneous (s.c.) dose of naloxone, that evidences the involvement of opioid receptors. This observation, coupled with previous reports that MSH/ACTH fragments may attenuate morphine-induced analgesia, suggests that melanocortins and endorphins can have opposite effect on nociception. It is possible that α-MSH and related peptides may be endogenous anti-opiates. Similar results were presented by Bertolini [135] as early as in 1979, who demonstrated that the injection of $ACTH_{1-24}$ (20–50 μg, i.c.v.) into the cerebral ventricles in rats reduced the reaction time in the hot plate test and nociception threshold in the tail-stimulation test. By this time, it was proposed that ACTH peptides could play a physiological role in nociception. ACTH at intermediate doses (0.5 and 1 μg, i.c.v.) caused hyperaglesia as indicated by a decrease in ear withdrawal latency in rabbits [136]. However, it has been demonstrated that ACTH at similar doses had no effect on nociception evaluated by tail-flick test when administered into the fourth ventricle of rats [137]. Interestingly, there are few reports, in which melanocortins are claimed to produce hypoalgesia. α-MSH (0.1–10 μg, i.c.v.) treatment in mice resulted in analgesia in the hot-plate test [138], and it has also been reported that α-MSH microinjections in PAG caused a reduction of response to pain [139].

The postulated role of melanocortin peptides and their receptors is even enhanced due to the suggested functional interaction of the melanocortins with the opioid system. α-MSH administered icv prior to morphine injection antagonized morphine-induced analgesia at doses lower than that necessary to produce hyperalgesia [140]. Progress in the identification and characterization of melanocortin receptor types have provided novel tools for the studies of interactions between melanocortins and addiction. The development of morphine tolerance and dependence can be inhibited by α-MSH [141]. Melanocortins can also attenuate the acquisition of heroin self-administration [142] and counteract opiate addiction [143], as it has been demonstrated by the induction of withdrawal-like symptoms in morphine-dependent rats [135].

Treatment of pain continues to be a troublesome clinical problem. Typically, pain appears as a result of the injury. However, in some cases, pain can become persistent, particularly as a result of a damage to the CNS. Damage or dysfunction in the CNS can lead to neuropathic pain. It may also results from the damage to the peripheral nerves, DRG or spinal cord. Conditions associated with neuropathic pain often

include components which are less responsive to typical analgesics treatment, i.e. opioids. Therefore, many studies have been undertaken to search for new potent analgesics which would be of great benefit to clinical practice. MSH/ACTH-like peptides possess neurotrophic activities. Their beneficial effects on regeneration of the damaged central and peripheral nerve tissue have been described. Melanocortin peptides may also be clinically effective and may have important potential in preventing peripheral neuropathies. Colocalization of opioid and melanocortin receptor expression, especially at the spinal cord level in the dorsal horn and in the gray matter surrounding the central canal suggests that melanocortins might play a role in nociceptive processes. The hypothesis of functional antagonistic interactions between the system of opioids and melanocortins is based on the observation that α-MSH and β-endorphin have a common precursor, POMC. After the products of the POMC gene are adequately processed, α-MSH and β-endorphin are stored in the same synaptic vesicles. Consequently, the release of α-MSH is always accompanied by a simultaneous and anatomically co-localized release of β-endorphins. Such a phenomenon has been observed in the cells of the locus coeruleus (LC), where α-MSH and β-endorphins regulate the activity of adenylate cyclase in the opposite manner: opioid receptors are negatively while melanocortin receptors are positively linked with the activity of adenylate cyclase [144]. In neurons receiving input through opioid and melanocortin receptors, AC might function as a recipient of signals from both systems which are concurrent to each other. MC receptor blockade attenuates a tonic action of α-MSH on nociception, allowing the analgesic effect of β-endorphin to develop, resulting in alleviation of allodynia [145].

The interactions between melanocortin and opioid systems at the level of the spinal cord are an example of physiological systems, cooperating with each other to modulate perception of pain. Out of all five melanocortin receptors, only MC3 and MC4 receptors are expressed in the nervous system. In comparison with MC3, the MC4 receptor is the only type, whose mRNA has been detected at the spinal cord level. The presence of melanocortin receptors in the regions important for conveying the nociceptive information was shown by analysis of the binding of a synthetic analogue of MSH (^{125}I-NDP-MSH) to the CNS structures. The expression of melanocortin receptors was shown in PAG, in the lamina X around the central canal, and dorsal horn (lamina I and II). In the spinal cord, expression of MC4 receptor was observed in the same regions as expression of the peptides originating from POMC (α-MSH/ACTH) [146]. Since the regions in which this co-expression occurs are important for nociception, we hypothesize that at the level of spinal cord, the melanocortin system is engaged in transmission of nociceptive information. However, the POMC mRNA expression at the spinal cord level remains still controversial. Some authors demonstrated mRNA encoding this melanocortin precursor protein [122,146], although it is rather believed that the descending pathways from the NTS are the main source of POMC. In contrast, the presence of the functional melanocortin receptors at the spinal level has been evidenced. In the animals with permanently injured sciatic nerves, the results of in situ binding of synthetic ligand of the melanocortin receptor (^{125}I-NDP-MSH) indicate an increase in the level of melanocortin receptors at the L4–L6 lumbar level of the spinal cord [147]. However, according to the work of van der Kraan [146], this change is not caused by the injury to the sciatic nerve per se, because the injury does not result in significant changes in the binding of ^{125}I-NDP-MSH in relation to the sham operated animals.

Since the first melanocortin receptors were cloned, following the discovery of specific binding sites for melanocortins in the central nervous system, selective compounds have become available to study the mechanism underlying the physiological role attributed to melanocortins. SHU9119 and MTII were applied to determine the role of melanocortin system in neuropathic pain. The team of W. H. Gispen performed the first works that confirmed the analgesic action of melanocortin receptor antagonist. They

published data indicating that the administration of SHU9119 into the cisterna magna reduces allodynia in the models of neuropathic pain, while the administration of melanocortin receptor agonist intensifies the pain caused by an injury to the sciatic nerve [147]. After permanent injury to the sciatic nerve, there is an increase in the binding of ^{125}I-NDP-MSH to the spinal cord (lamina I and II) suggesting elevation of the level of melanocortin receptors [147].

The studies performed in our laboratory also suggest that intrathecal (i.th.) administration of SHU9119, MC receptor antagonist, causes a decrease in cold and mechanical allodynia as well as lowers susceptibility to painful stimuli by blocking the MC4 receptor in the spinal cord [148]. On the other hand, the administration of MC receptor agonist MTII (i.th.), has opposite effect and causes an increase in cold and mechanical allodynia. In short, melanocortins have a pain-eliciting activity and eliminate the anesthetic action of morphine. The anti-allodynic effect of SHU9119 can be accomplished by a blockade of the influence of endogenous melanocortin system on the nociceptive information flow through MC4 receptor in the spinal cord. Therefore, the modulation of melanocortin system activity can influence the level of sensitivity to the painful stimuli. This is the reason justifying our interest in the melanocortin system, its involvement in conveying nociceptive information, and its role as a potent target in treating neuropathic pain. It is possible that the changes in the endogenous melanocortin system at the level of spinal cord are responsible for the pain syndromes caused by lesion to the peripheral nerves. The administration of SHU9119, brings about a noteworthy anti-allodynic effect in the tests examining animals' response to the lowered temperature or mechanical stimulation. After the administration of MTII, the animals react to the same test stimuli with increased sensitivity thus in a manner opposite to animals receiving SHU9119. The administration of D-Tyr-MTII, melanocortin receptor antagonist with higher selectivity for the MC4 than MC3 receptor, yielded similar results [147]. In short, the recently obtained data indicate that the MC4 receptor antagonists have great potential to play a valuable role in the therapy of neuropathic pain and the continuation of our experiments will bring us a step closer to decoding this mechanism. However, one should remember that melanocortin receptor antagonists used as potential analgesics can have side effects manifesting themselves as a changes in body weight because the MC4 receptor plays a role in maintaining metabolic balance of the body [35].

In summary, repeated administrations of MC4 receptor antagonists, such as SHU9119, into the spinal cord cause notable analgesic effect in rats with the injury to the sciatic nerve. It can be suggested that this effect is mediated by MC4 receptors present in the spinal cord. SHU9119 brings about satisfactory effect by reducing reaction to painful stimuli associated with the neuropathic pain syndrome without affecting a proper perception of pain in the uninjured paw of the animal. When morphine is used in the treatment of the symptoms of neuropathy, its analgesic effect is weaker than in other kinds of conditions associated with pain, which directed our attention towards interactions between opioids and melanocortins, that may contribute to this phenomenon. Currently, we postulate the importance of modulation of melanocortins system, comprising the peptides originating from the same prohormone as opioids, POMC, in alleviating the symptoms associated with the development of neuropathy.

Conclusions

We have reviewed some aspects of a very large and expanding range of research on melanocortin peptides. At present, one of the problems inspiring the widest interest is the role of melanocortins in the regulation of immune system. The activation of melanocortin receptors on glial and peripheral immune

cells modulates inflammatory responses and it should promote the control of inflammatory diseases. α-MSH analogues may aid the treatment of a variety of CNS and peripheral inflammatory disorders in which infection and inflammation coexist, as they combine antipyretic, anti-inflammatory, and antimicrobial effects.

Cloning of the melanocortin receptors in the early 1990s made possible to identify their synthetic ligands. Antagonists of MC receptors are useful tools for investigating the molecular mechanisms underlying melanocortin-associated effects. Better understanding of these mechanisms can potentially lead to new therapeutic opportunities in the treatment of inflammatory conditions and pain. The α-MSH is the candidate for a powerful drug, due to its pleiotropic effects on inflammation and energy homeostasis, which has a potential of therapeutic applications for the treatment of inflammatory diseases [149]. Since the pharmacokinetics of peptide delivery is not favorable from the pharmaceutical perspective, recent research has focused on plasmid-based vectors that constitutively express the immunomodulatory peptide α-MSH. The fusion constructs encode the α-MSH in frame with the first domain of serum albumin, separated by a linker and furin cleavage sites. The activity of these vectors provides tools and the impetus for testing the constructs in several animal models of chronic inflammation. The α-MSH has shown efficacy in many major animal models of inflammation, such as inflammatory bowel disease, rheumatoid arthritis, and localized inflammation. The peptide's anti-inflammatory activity extends to many regions of the body, including liver, brain, gut and skin [56,150–153]. Administration of large doses of the α-MSH in vivo has demonstrated that the peptide is well tolerated both in animals and humans [154–156]. Administration of plasmid-based vector which results in long-lasting expression of the peptide, helps to avoid repeated drug injection over short period of time in animal models or humans [157,158]. This strategy offers the advantages of sustained peptide expression from an expression vector that produces bioactive α-MSH [149]. The albumin-α-MSH-expressing constructs are currently being tested in various mouse models of inflammation and autoimmunity to evaluate their therapeutic potential. Ichiyama et al. [159] have published a report describing an expression vector encoding a signal sequence/α-MSH peptide which partially inhibits NF-κB activity in transfected glioma cells [159]. It should be possible to treat neurodegenerative disease, stroke, encephalitis, trauma, and other CNS disorders that have an inflammatory component through gene therapy with α-MSH vector [159].

Clarification of the role of the melanocortin system in the development of neuropathic pain and explanation of the relationship between melanocortins and opioid system will play a significant role in expanding our knowledge about the mechanisms of melanocortin action in chronic pain. Explaining the mechanisms that govern the changes resulting from the injury to the peripheral nerves can help us to develop new pharmacological strategies for approaching neuropathic pain. The mechanism of weakened action of intrathecally administered morphine in animals with neuropathic pain is still not clear. We have confidence that the explanation of interconnected actions of melanocortins and opioids will bring about valuable answers and open new perspectives in the search for effective treatment for patients with chronic pain.

Acknowledgements

This research was supported by statutory funds from the State Committee for Scientific Research (KBN; Warsaw).

References

[1] Eipper BA, Mains RE. Structure and biosynthesis of pro-opiomelanocortin/endorpin and related peptides. Endocrine Reviews 1980;1:1–27.
[2] Nakanishi S, Inoue A, Kita T, Nakamura M, Chang AC, Cohen SN, Numa S. Nucleotide sequence of cloned cDNA for bovine corticotropin-beta-lipotropin precursor. Nature 1979;278(5703):423–7.
[3] Roberts JL. Opioids I. In: Herz A, editor. Berlin: Springer-Verlag; 1993. p. 347–78.
[4] De Wied D, Jolles J. Neuropeptides derived from pro-opiocortin: behavioral, physiological, and neurochemical effects. Physiological Reviews 1982;62(3):976–1059.
[5] Wikberg JES. Melanocortin receptors: perspectives for novel drugs. European Journal of Pharmacology 1999; 375(1–3):295–310.
[6] Strand FL, Zuccarelli LA, Williams KA, Lee SJ, Lee TS, Antonawich FJ, Alves SE. Melanotropins as growth factors. Annals of the New York Academy of Sciences 1993;680:29–50.
[7] Korner J, Chun J, Harter D, Axel R. Isolation and functional expression of a mammalian prohormone processing enzyme, murine prohormone convertase 1. Proceedings of National Academy of Sciences USA 1991;88(15):6834–8.
[8] Zhou A, Mains RE. Endoproteolytic processing of proopiomelanocortin and prohormone convertases 1 and 2 in neuroendocrine cells overexpressing prohormone convertases 1 or 2. The Journal of Biological Chemistry 1994;269(26): 17440–7.
[9] Abdel-Malek ZA. Melanocortin receptors: their functions and regulation by physiological agonists and antagonists. Cellular and Molecular Life Sciences 2001;58(3):434–41.
[10] Eberle AN. The melanotropins. Basel: Karger; 1988.
[11] Mountjoy KG, Robins LS, Mortrud MT, Cone RD. The cloning of a family of genes that encode the melanocortin receptors. Science 1992;257(5074):1248–51.
[12] Chhajlani V, Wikberg JE. Molecular cloning and expression of the human melanocyte stimulating hormone receptor cDNA. FEBS Letters 1992;309(3):417–20.
[13] Gantz I, Konda Y, Tashiro T, Shimoto Y, Miwa H, Munzert G, Watson SJ, DelValle J, Yamada T. Molecular cloning of a novel melanocortin receptor. Journal of Biological Chemistry 1993;268(11):8246–50.
[14] Gantz I, Miwa H, Konda Y, Shimoto Y, Tashiro T, Watson SJ, DelValle J, Yamada T. Molecular cloning, expression, and gene localization of a fourth melanocortin receptor. Journal of Biological Chemistry 1993;268(20):15174–9.
[15] Chhajlani V, Muceniece R, Wikberg JE. Molecular cloning of a novel human melanocortin receptor. Biochemical and Biophysical Research Communications 1993;195(2):866–73.
[16] Konda Y, Gantz I, DelValle J, Shimoto Y, Miwa H, Yamada T. Interaction of dual intracellular signaling pathways activated by the melanocortin-3 receptor. Journal of Biological Chemistry 1994;269(18):13162–6.
[17] Buggy JJ. Binding of alpha-melanocyte-stimulating hormone to its G-protein-coupled receptor on B-lymphocytes activates the Jak/STAT pathway. The Biochemical Journal 1998;331(1):211–6.
[18] Mountjoy KG. The human melanocyte stimulating hormone receptor has evolved to become "super-sensitive" to melanocortin peptides. Molecular Endocrinology 1994;102(1–2):7–11.
[19] Sawyer TK, Sanfilippo PJ, Hruby VJ, Engel MH, Heward CB, Burnett JB, Hadley ME. 4-Norleucine, 7-D-phenylalanine-alpha-melanocyte-stimulating hormone: a highly potent alpha-melanotropin with ultralong biological activity. Proceedings of National Academy of Sciences USA 1980;77(10):5754–8.
[20] Star RA, Rajora N, Huang J, Stock RC, Catania A, Lipton JM. Evidence of autocrine modulation of macrophage nitric oxide synthase by alpha-melanocyte-stimulating hormone. Proceedings of National Academy of Sciences USA 1995; 92(17):8016–20.
[21] Hartmeyer M, Scholzen T, Becher E, Bhardwaj RS, Schwartz T, Luger TA. Human dermal microvascular endothelial cells express the melanocortin receptor type 1 and produce increased levels of IL-8 upon stimulation with alpha-melanocyte-stimulating hormone. Journal of Immunolog 1997;159(4):1930–7.
[22] Catania A, Rajora N, Capsoni F, Minonzio F, Star RA, Lipton JM. The neuropeptide alpha-MSH has specific receptors on neutrophils and reduces chemotaxis in vitro. Peptides 1996;17(4):675–9.
[23] Boston BA, Cone RD. Characterization of melanocortin receptor subtype expression in murine adipose tissues and in the 3T3-L1 cell line. Endocrinology 1996;137(5):2043–50.
[24] Wong KY, Rajora N, Boccoli G, Catania A, Lipton JM. A potential mechanism of local anti-inflammatory action of

alpha-melanocyte-stimulating hormone within the brain: modulation of tumor necrosis factor-alpha production by human astrocytic cells. Neuroimmunomodulation 1997;4(1):37–41.
[25] Luger TA, Scholzen T, Brzoska T, Becher E, Slominski A, Paus R. Cutaneous immunomodulation and coordination of skin stress responses by alpha-melanocyte-stimulating hormone. Annals of the New York Academy of Sciences 1998; 840:381–94.
[26] Chhajlani V. Distribution of cDNA for melanocortin receptor subtypes in human tissues. Biochemistry and Molecular Biology International 1996;38(1):73–80.
[27] Xia Y, Wikberg JE. Localization of ACTH receptor mRNA by in situ hybridization in mouse adrenal gland. Cell and Tissue Research 1996;286(1):63–8.
[28] Slominski A, Ermak G, Mihm M. ACTH receptor, CYP11A1, CYP17 and CYP21A2 genes are expressed in skin. The Journal of Clinical Endocrinology and Metabolism 1996;81(7):2746–9.
[29] Boston BA. The role of melanocortins in adipocyte function. Annals of the New York Academy of Sciences 1998;885: 75–84.
[30] Roselli-Rehfuss L, Mountjoy KG, Robbins LS, Mortrud MT, Low MJ, Tatro JB, Entwistle ML, Simerly RB, Cone RD. Identification of a receptor for gamma melanotropin and other proopiomelanocortin peptides in the hypothalamus and limbic system. Proceedings of National Academy of Sciences USA 1993;90(19):8856–60.
[31] Low MJ, Simerly R, Cone RD. Receptors for the melanocortin peptides in the central nervous system. Current Opinion in Endocrinol Diab 1994;1:79–88.
[32] Lindblom J, Schioth HB, Larsson A, Wikberg JE, Bergstrom L. Autoradiographic discrimination of melanocortin receptors indicates that the MC3 subtype dominates in the medial rat brain. Brain Research 1998;810(1–2): 161–71.
[33] Alvaro JD, Tatro JB, Quillan JM, Fogliano M, Eisenhard M, Lerner MR, Nestler EJ, Duman RS. Morphine down-regulates melanocortin-4 receptor expression in brain regions that mediate opiate addiction. Molecular Pharmacology 1996;50(3):583–91.
[34] Wikberg JE, Muceniece R, Mandrika I, Prusis P, Lindblom J, Post C, Skottner A. New aspects on the melanocortins and their receptors. Pharmacological Research 2000;42(5):393–420.
[35] Mountjoy KG, Wong J. Obesity, diabetes and functions for proopiomelanocortin-derived peptides. Molecular endocrinology Molecular and Cellular Endocrinology 1997;128(1–2):171–7.
[36] Labbe O, Desarnaud F, Eggerickx D, Vassart G, Parmentier M. Molecular cloning of a mouse melanocortin 5 receptor gene widely expressed in peripheral tissues. Biochemistry 1994;33(15):4543–9.
[37] Chen W, Kelly MA, Opitz-Araya X, Thomas RE, Low MJ, Cone RD. Exocrine gland dysfunction in MC5-R-deficient mice: evidence for coordinated regulation of exocrine gland function by melanocortin peptides. Cell 1997;91(6): 789–98.
[38] van der Kraan M, Adan RA, Entwistle ML, Gispen WH, Burbach JP, Tatro JB. Expression of melanocortin-5 receptor in secretory epithelia supports a functional role in exocrine and endocrine glands. Endocrinology 1998;139(5): 2348–55.
[39] Gantz I, Shimoto Y, Konda Y, Miwa H, Dickinson CJ, Yamada T. Molecular cloning, expression, and characterization of a fifth melanocortin receptor. Biochemical and Biophysical Research Communications 1994;200(3): 1214–20.
[40] Griffon N, Mignon V, Facchinetti P, Diaz J, Schwartz JC, Sokoloff P. Molecular cloning and characterization of the rat fifth melanocortin receptor. Biochemical and Biophysical Research Communications 1994;200(2):1007–14.
[41] Chen W. The melanocortin-5 receptor. In: Cone RD, editor. The Melanocortin receptors. Totowa, NJ: Humana; 2000. p. 449–72.
[42] Sawyer TK, Sanfilippo PJ, Hruby VJ, Engel MH, Heward CB, Burnett JB, Hadley ME. 4-Norleucine, 7-D-phenylalanine-alpha-melanocyte-stimulating hormone: a highly potent alpha-melanotropin with ultralong biological activity. Proceedings of National Academy of Sciences USA 1980;77(10):5754–8.
[43] Al-Obeidi F, Castrucci AM, Hadley ME, Hruby VJ. Potent and prolonged acting cyclic lactam analogues of alpha-melanotropin: design based on molecular dynamics. Journal of Medicinal Chemistry 1989;32(12):2555–61.
[44] Hadley ME, Marwan MM, al.-Obeidi F, Hruby VJ, Castrucci AM. Linear and cyclic alpha-melanotropin [4–10]-fragment analogues that exhibit superpotency and residual activity. Pigment cell research/sponsored by the European Society for Pigment Cell Research and the International Pigment Cell Society 1989;2(6):478–84.
[45] Bednarek MA, Macneil T, Kalyani RN, Tang R, Van der Ploeg LH, Weinberg DH. Analogs of MTII, lactam derivatives

of alpha-melanotropin, modified at the N-terminus, and their selectivity at human melanocortin receptors 3, 4, and 5. Biochemical and Biophysical Research Communications 1999;261(1):209–13.
[46] Abou-Mohamed G, Papapetropoulos A, Ulrich D, Catravas JD, Tuttle RR, Caldwell RW. HP-228, a novel synthetic peptide, inhibits the induction of nitric oxide synthase in vivo but not in vitro. Journal of Pharmacology and Experimental Therapeutics 1995;275(2):584–91.
[47] Hurby VJ, Lu D, Sharma SD, Castrucci AL, Kesterson RA, al.-Obeidi FA, Hadley ME, Cone RD. Cyclic lactam alpha-melanotropin analogues of Ac-Nle4-cyclo[Asp5, D-Phe7,Lys10] alpha-melanocyte-stimulating hormone-(4–10)-NH2 with bulky aromatic amino acids at position 7 show high antagonist potency and selectivity at specific melanocortin receptors. Journal of Medicinal Chemistry 1995;38(18):3454–61.
[48] Schioth HB, Mutulis F, Mucience R, Prusis P, Wikberg JE. Discovery of novel melanocortin4 receptor selective MSH analogues. British Journal of Pharmacology 1998;124(1):75–82.
[49] Li SJ, Varga K, Archer P, Hruby VJ, Sharma SD, Kesterson RA, Cone RD, Kunos G. Melanocortin antagonists define two distinct pathways of cardiovascular control by alpha- and gamma-melanocyte-stimulating hormones. Journal of Neuroscience 1996;16(16):5182–8.
[50] Fan W, Boston BA, Kesterson RA, Hurby VJ, Cone RD. Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. Nature 1997;385(6612):165–8.
[51] Kask A, Rago L, Mutulis F, Pahkla R, Wikberg JE, Schioth HB. Selective antagonist for the melanocortin 4 receptor (HS014) increases food intake in free-feeding rats. Biochemical and biophysical research communications 1988;245(1): 90–3.
[52] Kask A, Rago L, Korrovits P, Wikberg JE, Schioth HB. Evidence that orexigenic effects of melanocortin 4 receptor antagonist HS014 are mediated by neuropeptide Y. Biochemical and Biophysical Research Communications 1998; 248(2):245–9.
[53] Kask A, Mutulis F, Muceniece R, Pahkla R, Mutule I, Wikberg JE, Rago L, Schioth HB. Discovery of a novel superpotent and selective melanocortin-4 receptor antagonist (HS024): evaluation in vitro and in vivo. Endocrinology 1998; 139(12):5006–14.
[54] Schioth HB, Muceniece R, Wikberg JE. Characterisation of the melanocortin 4 receptor by radioligand binding. Pharmacology and toxicology 1996;79(3):161–5.
[55] Luger TA, Scholzen T, Grabbe S. The role of alpha-melanocyte-stimulating hormone in cutaneous biology. The journal of investigative dermatology. Symposium proceedings/the Society for Investigative Dermatology, Inc. [and] European Society for Dermatological Research (Review) 1997;2(1):87–93.
[56] Rajora N, Boccoli G, Catania A, Lipton JM. Alpha-MSH modulates experimental inflammatory bowel disease. Peptides 1997;18(3):381–5.
[57] Ceriani G, Diaz J, Murphree S, Catania A, Lipton JM. The neuropeptide alpha-melanocyte-stimulating hormone inhibits experimental arthritis in rats. Neuroimmunomodulation 1994;1(1):28–32.
[58] Rajora N, Boccoli G, Burns D, Sharma S, Catania AP, Lipton JM. Alpha-MSH modulates local and circulating tumor necrosis factor-alpha in experimental brain inflammation. Journal of Neuroscience 1997;17(6):2181–6.
[59] Huh SK, Lipton JM, Batjer HH. The protective effects of alpha-melanocyte stimulating hormone on canine brain stem ischemia. Neurosurgery 1997;40(1):132–9.
[60] Ichiyama T, Sakai T, Catania A, Barsh GS, Furukawa S, Lipton JM. Systematically administred alpha-melanocyte-stimulating peptides inhibit NF-κB activation in experimental brain inflammation. Brain Research 1999;836(1–2): 31–7.
[61] Chiao H, Kohda Y, McLeroy P, Craig L, Housini I, Star RA. Alpha-melanocyte-stimulating hormone protects against renal injury after ischemia in mice and rats. The Journal of Clinical Investigation 1997;99(6):1165–72.
[62] Ichiyama T, Sato S, Okada K, Catania A, Lipton JM. The neuroimmunomodulatory peptide alpha-MSH. Annals of the New York Academy of Sciences 2000;917:221–6.
[63] Kluger MJ, Kozak W, Leon LR, Soszynski D, Conn CA. Cytokines and fever. Neuroimmunomodulation 1995;2(4): 216–23.
[64] Kozak W, Kluger MJ, Tesfaigzi J, Kozak A, Mayfield KP, Wachulec M, Dokladny K. Molecular mechanisms of fever and endogenous antipyresis. Annals of the New York Academy of Sciences 2000;917:121–34.
[65] Strand FL. New vistas for melanocortins. Finally, an explanation for their pleiotropic functions. Annals of the New York Academy of Sciences 1999;897:1–16.
[66] Pittman QJ, Wilkinson MF. Central arginine vasopressin and endogenous antipyresis. Canadian Journal of Physiology and Pharmacology 1992;70(5):786–90.

[67] Catania A, Lipton JM. α-Melanocyte stimulating hormone in the modulation of host reactions. Endocrine Reviews 1993;14(5):564–76.
[68] Coelho MM, Souza GE, Pela IR. Endotoxin-induced fever is modulated by endogenous glucocorticoids in rats. The American Journal of Physiology 1992;263(2 Pt 2):423–7.
[69] Morrow LE, McClellan JL, Conn CA, Kluger MJ. Glucocorticoids alter fever and IL-6 responses to psychological stress and to lipopolysaccharide. The American Journal of Physiology 1993;264(5 Pt 2):1010–6.
[70] Lipton JM, Catania A. Anti-inflammatory actions of the neuroimmunomodulator alpha-MSH. Immunology Today 1997;18(3):140–5.
[71] Bell RC, Lipton JM. Pulsatile release of antipyretic neuropeptide alpha-MSH from septum of rabbit during fever. The American Journal of Physiology 1987;252(6 Pt 2):1152–7.
[72] Huang QH, Entwistle ML, Alvaro JD, Duman RS, Hruby VJ, Tatro JB. Antipyretic role of endogenous melanocortins mediated by central melanocortin receptors during endotoxin-induced fever. Journal of Neuroscience 1997; 17(9):3343–51.
[73] Huang QH, Hruby VJ, Tatro JB. Role of central melanocortins in endotoxin-induced anorexia. The American Journal of Physiology 1999;276(3 Pt 2):864–71.
[74] Hagan MM, Benoit SC, Rushing PA, Woods SC, Seeley RJ. Society for neuroscience abstracts. 30th Annual Meeting New Orleans, LA, November 4–9; 2000. p. 278.
[75] Luger TA, Kalden D, Scholzen TE, Brzoska T. Alpha-melanocyte-stimulating hormone as a mediator of tolerance induction. Pathobiology 1999;67(5–6):318–21.
[76] Lotti T, Bianchi B, Ghersetich I, Brazzini B, Hercogova J. Can the brain inhibit inflammation generated in the skin? The lesson of gamma-melanocyte-stimulating hormone. International Journal of Dermatology 2002;41(6): 311–8.
[77] Macaluso A, McCoy D, Ceriani G, Watanabe T, Biltz J, Catania A, Lipton JM. Antiinflammatory influences of alpha-MSH molecules: central neurogenic and peripheral actions. Journal of Neuroscience 1994;14(4):2377–82.
[78] Getting SJ, Gibbs L, Clark AJ, Flower RJ, Perretti M. POMC gene-derived peptides activate melanocortin type 3 receptor on murine macrophages, suppress cytokine release, and inhibit neutrophil migration in acute experimental inflammation. Journal of Immunology 1999;162(12):7446–53.
[79] Getting SJ, Allcock GH, Flower R, Perretti M. Natural and synthetic agonists of the melanocortin receptor type 3 possess anti-inflammatory properties. Journal of Leukocyte Biology 2001;69(1):98–104.
[80] Taherzadeh S, Sharma S, Chajlani V, Gantz I, Rajora N, Demitri MT, Kelly L, Zhao H, Ichiyama T, Catania A, Lipton JM. α-MSH and its receptors in regulation of tumor necrosis factor-α by human monocyte/macrophages. The American Journal of Physiology 1999;276(5 Pt 2):1289–94.
[81] Horn KD. Evolving strategies in the tratment of sepsis and systemic inflammtory response syndrome (SIRS). QJM: monthly journal of the Association of Physicians 1998;91:265–77.
[82] Luger TA, Brzoska T, Scholzen TE, Kalden DH, Sunderkotter C, Armstrong C, Ansel J. The role of alpha-MSH as a modulator of cutaneous inflammation. Annals of the New York Academy of Sciences 2000;917:232–8.
[83] Ghosh S, May MJ, Kopp EB. NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. Annual Review of Immunology 1998;16:225–60.
[84] Pahl HL. Activators and target genes of Rel/NF-kappaB transcription factors. Oncogene 1999;18(49):6853–66.
[85] Flohe L, Brigelius-Flohe R, Saliou C, Traber MG, Packer L. Redox regulation of NF-kappa B activation. Free Radical Biology and Medicine 1997;22(6):1115–26.
[86] Siebenlist U, Franzoso G, Brown K. Structure, regulation and function of NF-kappa B. Annual Review of Cell Biology 1994;10:405–55.
[87] Ichiyama T, Zhao H, Catania A, Furukawa S, Lipton JM. alpha-melanocyte-stimulating hormone inhibits NF-kappaB activation and IkappaBalpha degradation in human glioma cells and in experimental brain inflammation. Experimental Neurology 1999;157(2):359–65.
[88] Lipton JM, Catania A, Ichiyama T. Marshaling the Anti-Inflammatory Influence of the Neuroimmunomodulator alpha-MSH. News in physiological sciences: an international journal of physiology produced jointly by the International Union of Physiological Sciences and the American Physiological Society 2000;15:192–5.
[89] Kalden DH, Scholzen T, Brzoska T, Luger TA. Mechanisms of the antiinflammatory effects of alpha-MSH. Role of transcription factor NF-kappa B and adhesion molecule expression. Annals of the New York Academy of Sciences 1999;885:254–61.

[90] Butcher EC. Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. Cell 1991;67(6): 1033–6.
[91] Slominski A, Wortsman J, Luger T, Paus R, Solomon S. Corticotropin releasing hormone and proopiomelanocortin involvement in the cutaneous response to stress. Physiological Reviews 2000;80(3):979–1020.
[92] Catania A, Airaghi L, Garofalo L, Cutuli M, Lipton JM. The neuropeptide alpha-MSH in HIV infection and other disorders in humans. Annals of the New York Academy of Sciences 1998;840:848–56.
[93] Scholzen TE, Kalden DH, Brzoska T, Fisbeck T, Fastrich M, Schiller M, Bohm M, Schwarz T, Armstrong CA, Ansel JC, Luger TA. Expression of proopiomelanocortin peptides in human dermal microvascular endothelial cells: evidence for a regulation by ultraviolet light and interleukin-1. The journal of investigative dermatology. Symposium proceedings/the Society for Investigative Dermatology, Inc. [and] European Society for Dermatological Research 2000;115(6): 1021–8.
[94] Bohm M, Schiller M, Stander S, Seltmann H, Li Z, Brzoska T, Metze D, Schioth HB, Skottner A, Seiffert K, Zouboulis CC, Luger TA. Evidence for expression of melanocortin-1 receptor in human sebocytes in vitro and in situ. The journal of investigative dermatology. Symposium proceedings/the Society for Investigative Dermatology, Inc. [and] European Society for Dermatological Research 2002;118(3):533–9.
[95] Catania A, Cutuli M, Garofalo L, Carlin A, Airaghi L, Barcellini W, Lipton JM. The neuropeptide alpha-MSH in host defense. Ann NY Acad Sci 2000;917:227–31.
[96] Richards DB, Lipton JM. Effect of alpha-MSH 11–13 (lysine-proline-valine) on fever in the rabbit. Peptides 1984;5(4): 815–7.
[97] Hiltz ME, Lipton JM. Antiinflammatory activity of a COOH-terminal fragment of the neuropeptide alpha-MSH. The FASEB journal: official publication of the Federation of American Societies for Experimental Biology 1989;3(11): 2282–4.
[98] Cutuli M, Cristiani S, Lipton JM, Catania A. Antimicrobial effects of alpha-MSH peptides. Journal of Leukocyte Biology 2000;67(2):233–9.
[99] Bhattacharya A, Datta A. Effect of cyclic AMP on RNA and protein synthesis in Candida albicans. Biochemical and Biophysical Research Communications 1977;77(4):1483–544.
[100] Fauci AS. Host factors and the pathogenesis of HIV-induced disease. Nature 1996;384(6609):529–34.
[101] Poli G, Fauci AS. Cytokine modulation of HIV expression. Seminars in Immunology 1993;5(3):165–73.
[102] Rosenberg ZF, Fauci AS. Immunopathogenesis of HIV infection. The FASEB journal: official publication of the Federation of American Societies for Experimental Biology 1991;5(10):2382–90.
[103] Bhardwaj R, Becher E, Mahnke K, Hartmeyer M, Schwarz T, Scholzen T, Luger TA. Evidence for the differential expression of the functional alpha-melanocyte-stimulating hormone receptor MC-1 on human monocytes. Journal of Immunology 1997;158(7):3378–84.
[104] Artuc M, Grutzkau A, Luger T, Henz BM. Expression of MC1- and MC5-receptors on the human mast cell line HMC-1. Annals of the New York Academy of Sciences 1999;885:364–7.
[105] Edwards PM, Van der Zee CE, Verhaagen J, Schotman P, Jennekens FG, Gispen WH. Evidence that the neurotrophic actions of alpha-MSH may derive from its ability to mimick the actions of a peptide formed in degenerating nerve stumps. Journal of the Neurological Sciences 1984;64(3):333–40.
[106] Plantinga LC, Verhaagen J, Edwards PM, Hali M, Brakkee JH, Gispen WH. Pharmacological evidence for the involvement of endogenous alpha-MSH-like peptides in peripheral nerve regeneration. Peptides 1995;16(2):319–24.
[107] Verhaagen J, Edwards PM, Jennekens FG, Gispen WH. Pharmacological aspects of the influence of melanocortins on the formation of regenerative peripheral nerve sprouts. Peptides 1987;8(4):581–4.
[108] Adan RA, van der Kraan M, Doornbos RP, Bar PR, Burbach JP, Gispen WH. Melanocortin receptors mediate alpha-MSH-induced stimulation of neurite outgrowth in neuro 2A cells. Brain research. Molecular Brain Research 1996;36(1):37–44.
[109] Van der Neut R, Bar PR, Sodaar P, Gispen WH. Trophic influences of alpha-MSH and ACTH4–10 on neuronal outgrowth in vitro. Peptides 1988;9(5):1015–20.
[110] Daval JL, Louis JC, Gerard MJ, Vincendon G. Influence of adrenocorticotropic hormone on the growth of isolated neurons in culture. Neuroscience Letters 1983;36(3):299–304.
[111] Richter-Landsberg C, Jastorff B. The role of cAMP in nerve growth factor-promoted neurite outgrowth in PC12 cells. The Journal of cell biology 1986;102(3):821–9.
[112] Strand FL, Kung TT. ACTH accelerates recovery of neuromuscular function following crushing of peripheral nerve. Peptides 1980;1(2):135–8.

[113] Strand FL, Lee SJ, Lee TS, Zuccarelli LA, Antonawich FJ, Kume J, Williams KA. Non-corticotropic ACTH peptides modulate nerve development and regeneration. Reviews in the Neurosciences 1993;4(4):321–63.
[114] Lee SJ, Lee TS, Alves SE, Strand FL. Immunocytochemical localization of ACTH-(4–10) in the rat spinal cord following peripheral nerve trauma. Annals of the New York Academy of Sciences 1994;739:320–3.
[115] Antonawich FJ, Azmitia EC, Kramer HK, Strand FL. Specificity versus redundancy of melanocortins in nerve regeneration. Annals of the New York Academy of Sciences 1994;739:60–73.
[116] Attella MJ, Hoffman SW, Pilotte MP, Stein DG. Effects of BIM-22015, an analog of ACTH4–10, on functional recovery after frontal cortex injury. Behavioral and Neural Biology 1992;57(2):157–66.
[117] Wolterink G, Van Zanten E, Kamsteeg H, Radhakishun FS, Van Ree JM. Functional recovery after destruction of dopamine systems in the nucleus accumbens of rats. II. Facilitation by the ACTH-(4–9) analog ORG 2766. Brain Research 1990;507(1):101–8.
[118] de Koning P, Neijt JP, Jennekens FG, Gispen WH. Org.2766 protects from cisplatin-induced neurotoxicity in rats. Experimental neurology 1987;97(3):746–50.
[119] de Wied D. Neurotrophic effects of ACTH/MSH neuropeptides. Acta Neurobiologiae Experimentalis. (Warsz) 1990; 50(4–5):353–66.
[120] Joosten EA, Majewska B, Houweling DA, Bar PR, Gispen WH. Alpha-melanocyte stimulating hormone promotes regrowth of injured axons in the adult rat spinal cord. Journal of Neurotrauma 1999;16(6):543–53.
[121] Van der Zee CE, Nielander HB, Vos JP, Lopes da Silva S, Verhaagen J, Oestreicher AB, Schrama LH, Schotman P, Gispen WH. Expression of growth-associated protein B-50 (GAP43) in dorsal root ganglia and sciatic nerve during regenerative sprouting. Journal of Neuroscience 1989;9(10):3505–12.
[122] Plantinga LC, Verhaagen J, Edwards PM, Schrama LH, Burbach JP, Gispen WH. Expression of the pro-opiomelanocortin gene in dorsal root ganglia, spinal cord and sciatic nerve after sciatic nerve crush in the rat. Brain Research. Molecular Brain Research 1992;16(1–2):135–42.
[123] Verhaagen J, Edwards PM, Gispen WH. Damaged rat peripheral nerves do not contain detectable amounts of alpha-MSH. Journal of Neuroscience Research 1988;19(1):14–8.
[124] Hol EM, Gispen WH, Bar PR. ACTH-related peptides: receptors and signal transduction systems involved in their neurotrophic and neuroprotective actions. Peptides 1995;16(5):979–93.
[125] Rydel RE, Greene LA. cAMP analogs promote survival and neurite outgrowth in cultures of rat sympathetic and sensory neurons independently of nerve growth factor. Proceedings of National Academy of Sciences USA 1988; 85(4):1257–61.
[126] Pichichero M, Beer B, Clody DE. Effects of dibutyryl cyclic AMP on restoration of function of damaged sciatic nerve in rats. Science 1973;182(113):724–5.
[127] Kilmer SL, Carlsen RC. Forskolin activation of adenylate cyclase in vivo stimulates nerve regeneration. Nature 1984; 307(5950):455–7.
[128] Nakagawa-Yagi Y, Saito Y, Takada Y, Nakamura H. Suppressive effect of carbachol on forskolin-stimulated neurite outgrowth in human neuroblastoma NB-OK1 cells. Biochemical and Biophysical Research Communications 1992;182(1): 45–54.
[129] Roisen FJ, Murphy RA, Pichichero ME, Braden WG. Cyclic adenosine monophosphate stimulation of axonal elongation. Science 1972;175(17):73–4.
[130] Mattson MP, Taylor-Hunter A, Kater SB. Neurite outgrowth in individual neurons of a neuronal population is differentially regulated by calcium and cyclic AMP. Journal of Neuroscience 1988;8(5):1704–11.
[131] Lankford KL, Letourneau PC. Evidence that calcium may control neurite outgrowth by regulating the stability of actin filaments. The Journal of Cell Biology 1989;109(3):1229–43.
[132] al-Mohanna FA, Cave J, Bolsover SR. A narrow window of intracellular calcium concentration is optimal for neurite outgrowth in rat sensory neurones Brain Research. Developmental Brain Research 1992;70(2):287–90.
[133] Bijlsma WA, Schotman P, Jennekens FG, Gispen WH, De Wied D. The enhanced recovery of sensorimotor function in rats is related to the melanotropic moiety of ACTH/MSH neuropeptides. European Journal of Pharmacology 1983; 92(3–4):231–6.
[134] Sandman CA, Kastin AJ. Intraventricular administration of MSH induces hyperalgesia in rats. Peptides 1981;2(2): 231–3.
[135] Bertolini A, Poggioli R, Fratta W. Withdrawal symptoms in morphine-dependent rats intracerebroventricularly injected with ACTH1-24 and with beta-MSH. Life Sciences 1981;29(3):249–52.

[136] Williams Jr DW, Lipton JM, Giesecke Jr AH. Influence of centrally administered peptides on ear withdrawal from heat in the rabbit. Peptides 1986;7(6):1095–100.
[137] Smock T, Fields HL. ACTH1-24 blocks opiate-induced analgesia in the rat. Brain Research 1981;212(1):202–6.
[138] Ohkubo T, Shibata M, Takahashi H, Naruse S. Naloxone prevents the analgesic action of alpha-MSH in mice. Experientia 1985;41(5):627–8.
[139] Walker JM, Akil H, Watson SJ. Evidence for homologous actions of pro-opiocortin products. Science 1980;210(4475): 1247–9.
[140] Contreras PC, Takemori AE. Antagonism of morphine-induced analgesia, tolerance and dependence by alpha-melanocyte-stimulating hormone. Journal of Pharmacology and Experimental Therapeutics 1984;229(1):21–6.
[141] Szekely JI, Miglecz E, Dunai-Kovacs Z, Tarnawa I, Ronai AZ, Graf L, Bajusz S. Attenuation of morphine tolerance and dependence by alpha-melanocyte stimulating hormone(alpha-MSH). Life Scieces 1979;24(21):1931–8.
[142] van Ree JM. The influence of neuropeptides related to pro-opiomelanocortin on acquisition of heroin self-administration of rats. Life Sciences 1983;33(23):2283–9.
[143] Alvaro JD, Tatro JB, Duman RS. Melanocortins and opiate addiction. Life Sciences 1997;61(1):1–9.
[144] Rene F, Muller A, Jover E, Kieffer B, Koch B, Loeffler JP. Melanocortin receptors and delta-opioid receptor mediate opposite signalling actions of POMC-derived peptides in CATH.a cells. European Journal of Neuroscience 1998;10(5): 1885–94.
[145] Vrinten DH, Kalkman CJ, Adan RA, Gispen WH. Neuropathic pain: a possible role for the melanocortin system? Eurropean Journal of Pharmacology 2001;429(1–3):61–9.
[146] van der Kraan M, Tatro JB, Entwisltle ML, Brakkee JH, Burbach JP, Adan RA, Gispen WH. Expression of melanocortin receptors and pro-opiomelanocortin in the rat spinal cord in relation to neurotrophic effects of melanocortins. Brain Research. Molecular Brain Research 1999;63(2):276–86.
[147] Vrinten DH, Gispen WH, Groen GJ, Adan RAH. Antagonism of the melanocortin system reduces cold and mechanical allodynia in mononeurophatic rats. Journal of Neuroscience 2000;20(21):8131–7.
[148] Starowicz K, Przewlocki R, Gispen WH, Przewlocka B. Modulation of melanocortin-induced changes in spinal nociception by μ-opioid receptor agonist and antagonist in neuropathic rats. NeuroReport 2002;13(18):2447–52.
[149] Etemad-Moghadam B, Chen H, Yin P, Aziz N, Hedley ML. Inhibition of NF-kappa B activity by plasmid expressed alphaMSH peptide. Journal of Neuroimmunology 2002;125(1–2):23–9.
[150] Ceriani G, Diaz J, Murphree S, Catania A, Lipton JM. The neuropeptide alpha-melanocyte-stimulating hormone inhibits experimental arthritis in rats. Neuroimmunomodulation 1994;1(1):28–32.
[151] Chiao H, Foster S, Thomas R, Lipton J, Star RA. Alpha-melanocyte-stimulating hormone reduces endotoxin-induced liver inflammation. The Journal of Clinical Investigation 1996;97(9):2038–44.
[152] Lipton JM, Macaluso A, Hiltz ME, Catania A. Central administration of the peptide alpha-MSH inhibits inflammation in the skin. Peptides 1991;12(4):795–8.
[153] Rajora N, Boccoli G, Burns D, Sharma S, Catania AP, Lipton JM. Alpha-MSH modulates local and circulating tumor necrosis factor-alpha in experimental brain inflammation. Journal of Neuroscience 1997;17(6):2181–6.
[154] Gispen WH, Hamers FP, Vecht CJ, Jennekens FG, Neyt JP. ACTH/MSH like peptides in the treatment of cisplatin neuropathy. The Journal of Steroid Biochemistry and Molecular Biology 1992;43(1–3):179–83.
[155] Holdeman M, Lipton JM. Antipyretic activity of a potent alpha-MSH analog. Peptides 1985;6(2):273–5.
[156] Lerner AB, McGuire W. Effect of alpha- and beta-melanocyte stimulating hormone on skin colour of man. Nature 1961; 189:176–9.
[157] Dorr RT, Lines R, Levine N, Brooks C, Xiang L, Hruby VJ, Hadley ME. Evaluation of melanotan-II, a superpotent cyclic melanotropic peptide in a pilot phase-I clinical study. Life Sciences 1996;58(20):1777–84.
[158] Levine N, Sheftel SN, Eytan T, Dorr RT, Hadley ME, Weinrach JC, Ertl GA, Toth K, McGee DL, Hruby VJ. Induction of skin tanning by subcutaneous administration of a potent synthetic melanotropin. JAMA: the Journal of the American Medical Association 1991;266(19):2730–6.
[159] Ichiyama T, Campbell IL, Furukawa S, Catania A, Lipton JM. Autocrine alpha-melanocyte-stimulating hormone inhibits NF-kappaB activation in human glioma. Journal of Neuroscience Research 1999;58(5):684–9.